Troubleshooting

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Background

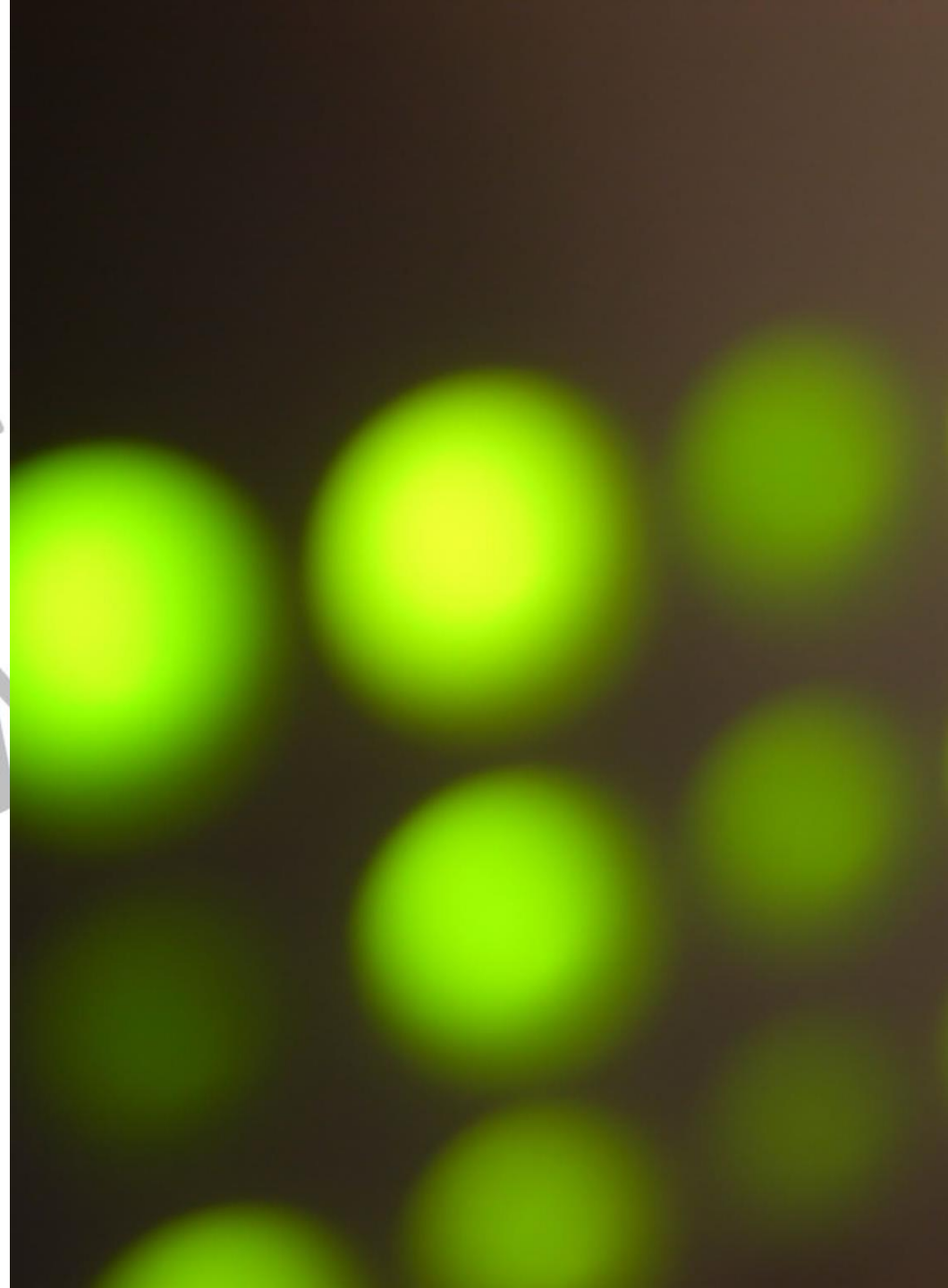
In October 2023, Towson University was awarded a cooperative agreement from NIST to develop a standardized DNA training curriculum for the United States that address the components in ANSI/ASB Standard 115, *Standards for Training in Forensic Short Tandem Repeat Typing Methods Using Amplification, DNA Separation, and Allele Detection*. 2020. 1st Ed.

This presentation addresses the knowledge-based portion of the training program and covers the topic outlined in 4.2.3i in ANSI/ASB Standard 115.

Learning Objectives

This material will provide trainees with an understanding of troubleshooting components, including:

1. thermal cycling errors (e.g., ramping, temperature control);
2. DNA detection errors (e.g., spectral calibration failure, resolution failure);
3. general equipment failure.



Terms & Definitions (ANSI/ASB 115)

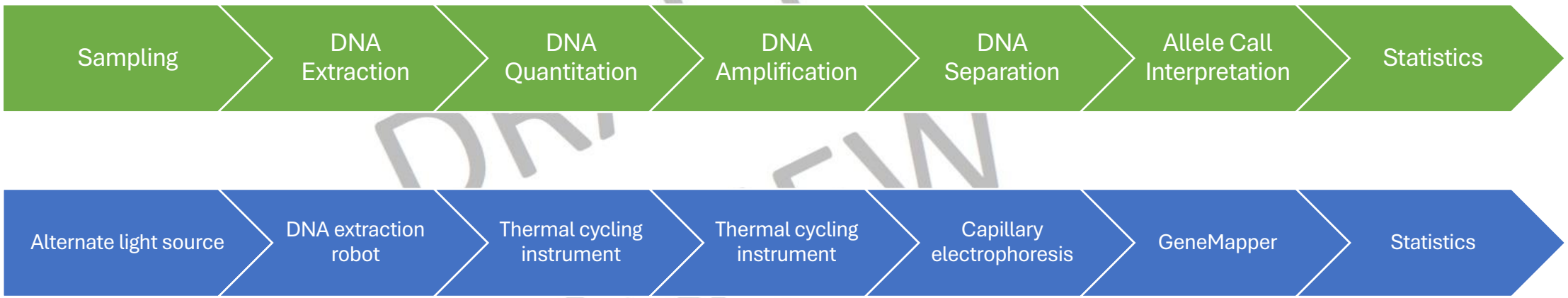
- **Artifact.** A non-allelic product of the amplification process (e.g., stutter, non-templated nucleotide addition, or other non-specific product), an anomaly of the detection process (e.g., pull-up or spike), or a byproduct of primer synthesis (e.g., “dye blob”) that may be observed on an electropherogram; some artifacts may complicate the interpretation of DNA profiles when they cannot be distinguished from the actual allele(s) from a particular sample.
- **Spectral calibration.** An examination of the contribution of overlap in the emission spectrum of fluorescent dyes used for a specific DNA test on a capillary electrophoresis instrument; permits the color deconvolution necessary for multi-color STR typing or sequencing to be performed; a poor spectral calibration may cause artifact peaks or inaccurate peak height determinations.
- **Stochastic.** 1) Chance, or random variation 2) in DNA testing, refers to random sampling error from extracts containing low levels of DNA and/or random variation in selection of alleles amplified at a particular locus.

Control Samples and Troubleshooting

Always include **positive** and **negative** controls in sample testing.

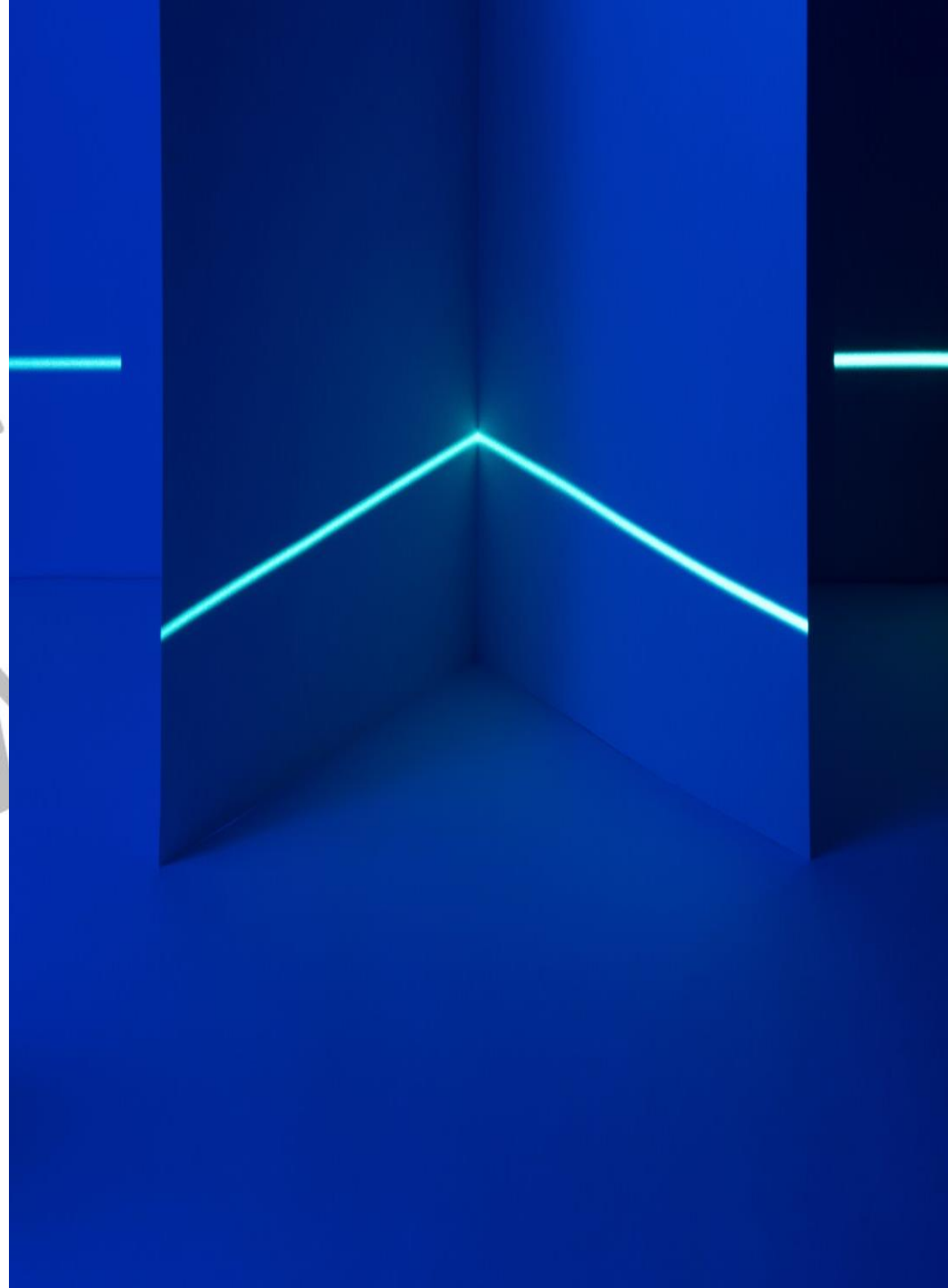
Controls will be used to determine if the method or instrument **performed properly.**

DNA Typing Process & Instrumentation



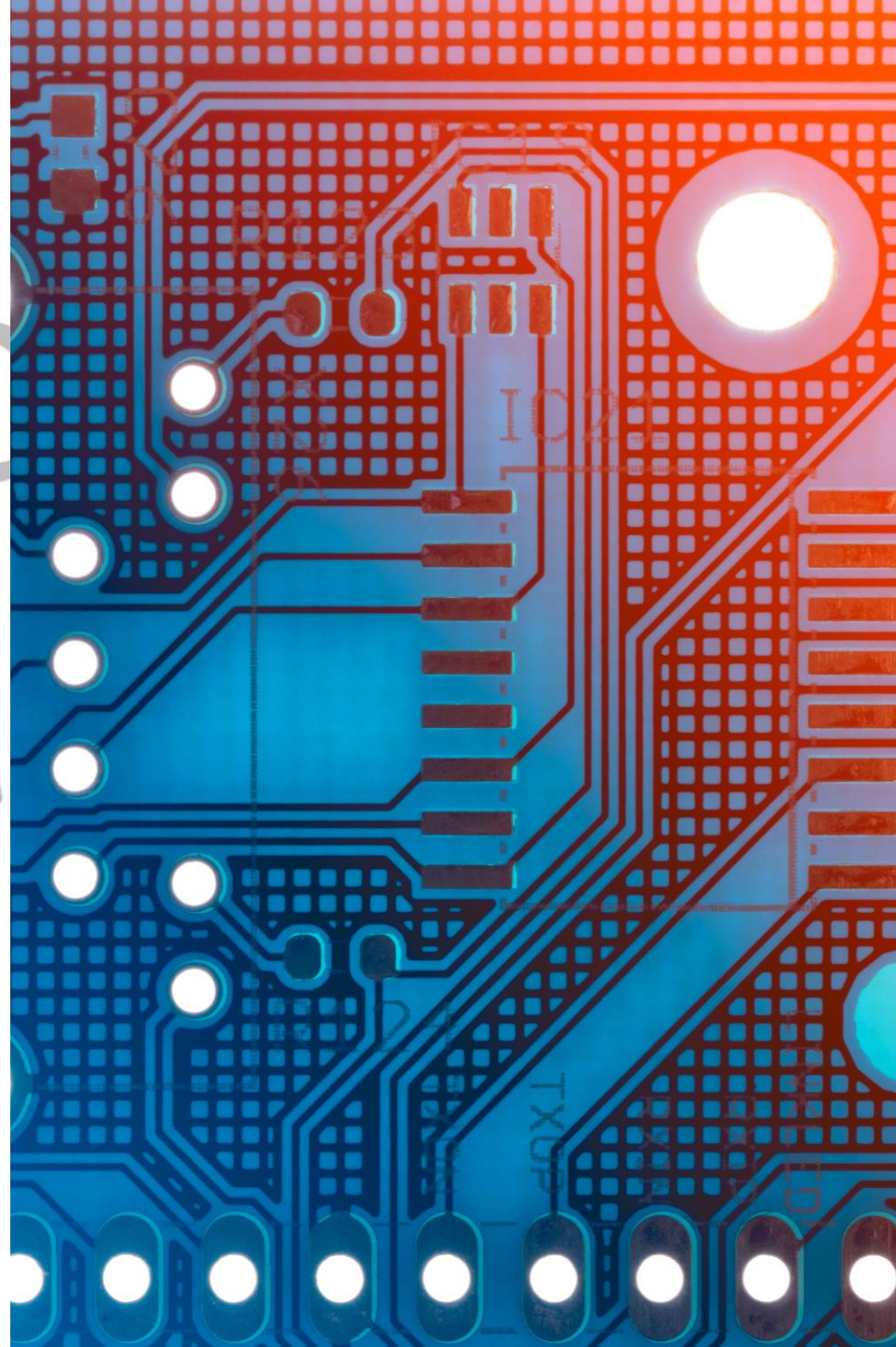
Interpretation: Thermal Cycling and CE DNA Detection Errors

- Control or sample issues
  - Positive controls and samples fail to amplify or lead to an unexpected result
  - Amplified DNA is not detected by real time detection system or incomplete cycling leads to low DNA yield
  - Positive controls and sample STRs will fail to separate or be detected



Thermal Cycler Failure

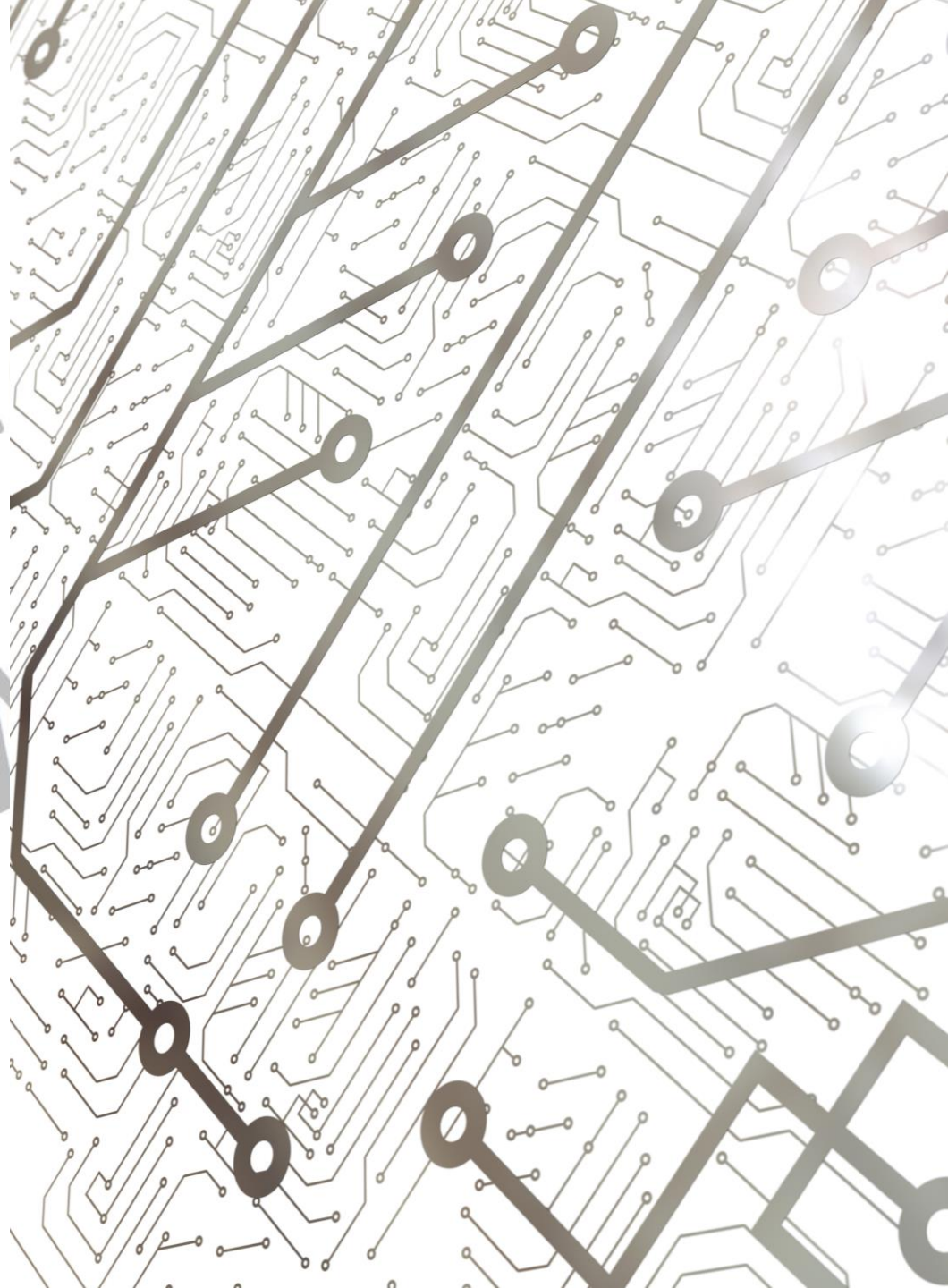
- Power / battery / surge
- Electronics failure from hot – cold cycling
  - Heater lid short
  - Circuit control board solder
- Reduced DNA amplification / yield
  - Heating / cooling rate too low
  - Faulty heating element (Peltier thermoelectric thermal control assembly)
- No screen display
  - Blown fuse



Veriti Thermal Cycler User Guide, Applied Biosystems, June 2010, https://assets.thermofisher.com/TFS-Assets/LSG/manuals/cms_042832.pdf

Thermal Cycling Error Response

- Check calibration and settings for temperature cycles and attainment
- Run instrument verification to check performance
- Check sensor error codes in manual and response
- Check lid seal: ajar or open lid can lead to an open circuit for the lid heater
- Decontaminate if contamination detected
- Use cotton swab with ethanol or isopropanol to clean lenses to improve detection sensitivity



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Rotor-Gene® Q MDx User Manual, v. 1, Qiagen, February 2018 , https://www.qiagen.com/us/resources/download.aspx?id=d0f79b96-f0b1-400e-8a0a-def2a6d19e42&lang=en

Thermal Cycling Errors: Temperature Control

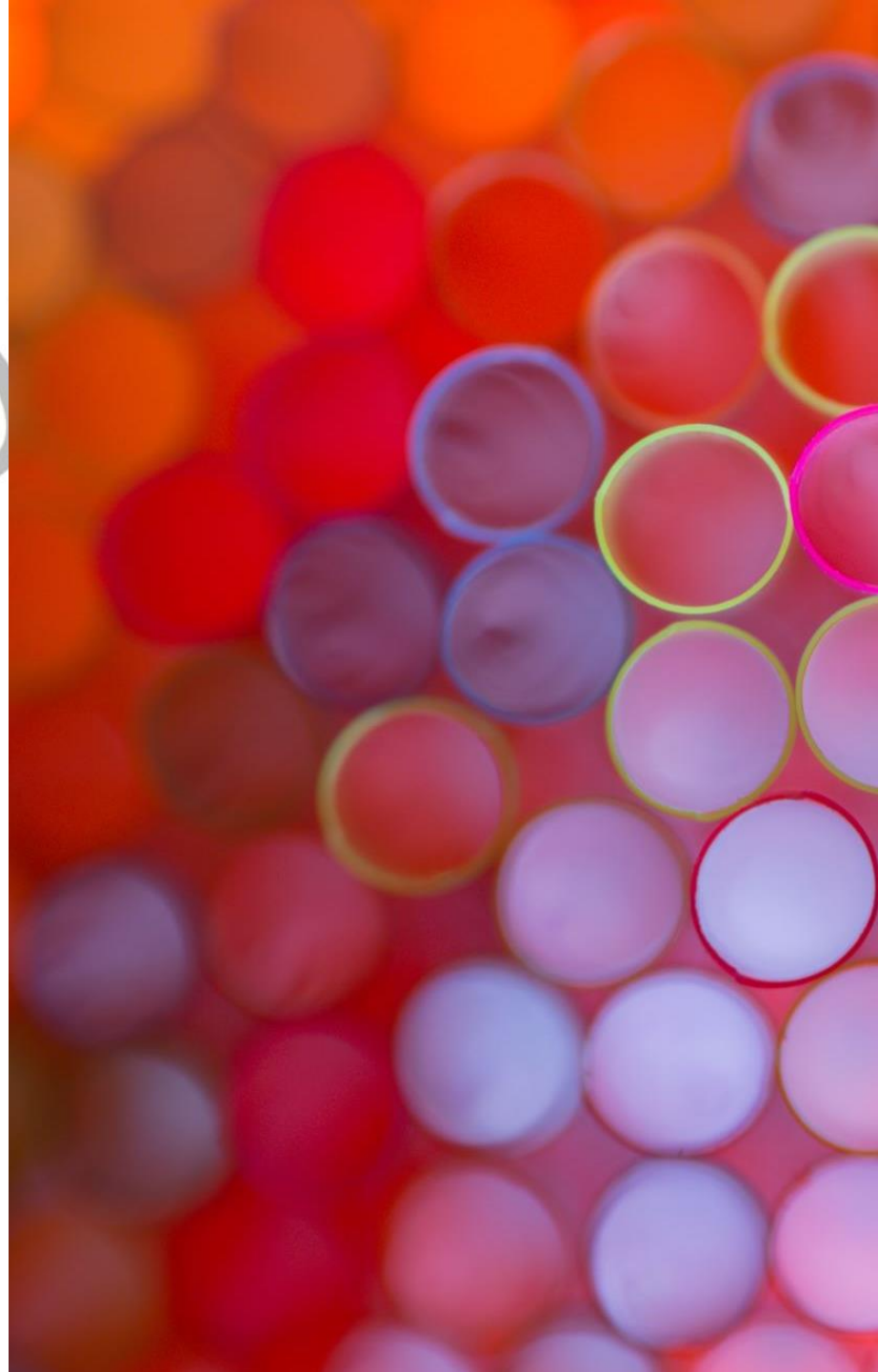
- Symptoms
  - Failure to amplify
  - Low product yield or detection
  - Non-specific target amplification
- Solutions
  - Check tube fit in wells
  - Use thin-walled and low-profile plastics
  - Use clear plastics for fluorescence detection
  - Do not exceed fill volume
  - Increase annealing temperature to increase specificity
  - Mix primers and master mix well and rerun

Thermal Cycling Errors: Ramping (Ramp Rate)

- The higher the ramp rate, the shorter the run time.
- **Symptoms** of ramping errors
  - Non-specific priming or spurious annealing
  - Overshooting denaturation temperature
- **Troubleshooting** ramping errors
  - Set ramp rate to highest speed to avoid non-specific priming
  - Slow down ramp rate to avoid overshooting temperature

Thermal Cycling Issues

- Melted plate or tubes
  - Incompatible consumables
- Fluorescent detection issues
  - Improper sample position may lead to a reduction in signal
  - Cloudy or dusty windows or lenses lead to low detection and underestimating quantitation values
  - Low laser intensity leads to low detection
  - Gain set too high leads to oversaturation while too low will lead to the signal being undetectable from the background noise



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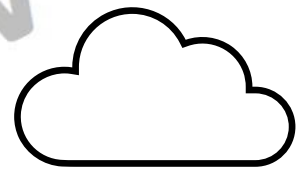
DNA Detection Errors: Spectral Calibration Failure

Leading to pull-up

- Rerun

Low intensity

- Cloudy detector window, clean off with cotton swab
- Replace laser or LED



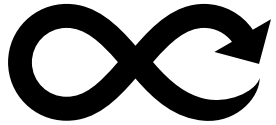
DNA Detection Errors: Resolution Failure

Poorly resolved peaks

- Change polymer and rerun

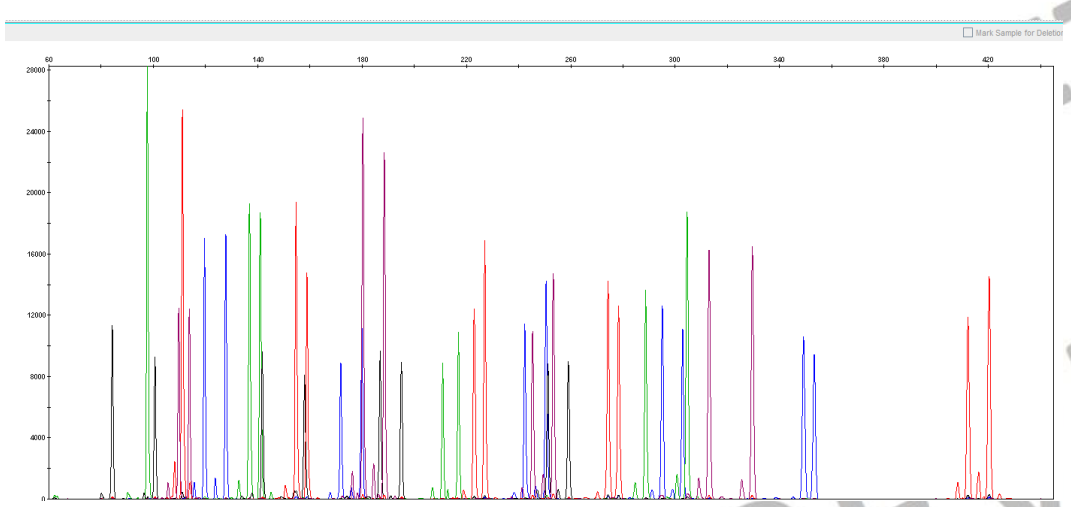
Secondary structure is forming, and migration is anomalous

- Run in Hi Di Formamide and heat and snap cool samples

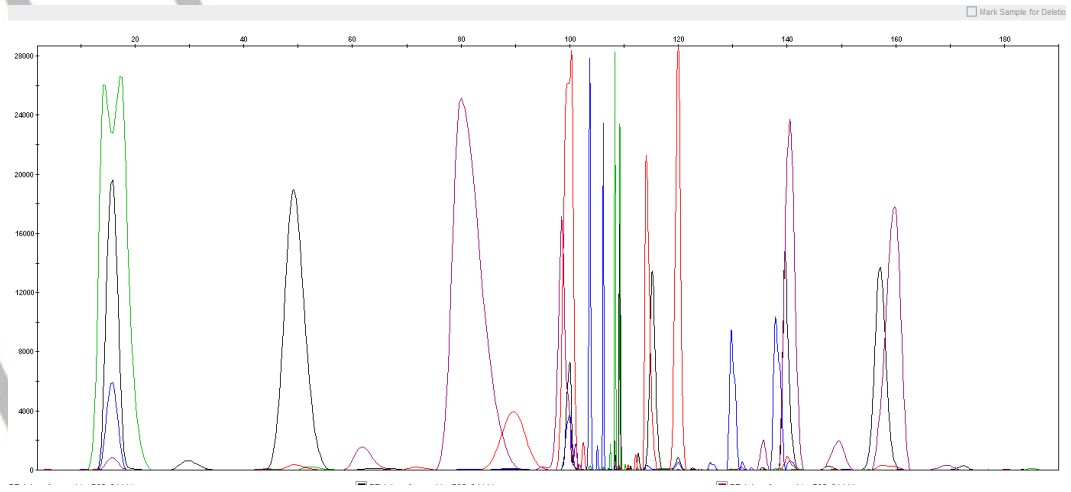


Example of Secondary Structure Formation

(With HiDi Formamide)



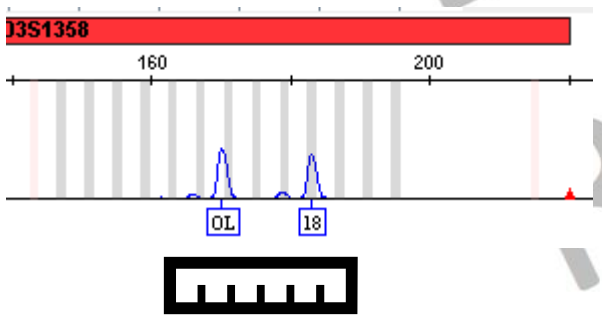
Without HiDi Formamide



DNA Interpretation: CE Run Issues

Off Ladder (OL)

- Ensure the correct size standard was used and mark the sizes in GeneMapper software
- Low CE room temperature or low buffer
- Rerun allelic ladder



Spikes

- Check for and remove bubbles and rerun
- Check that polymer remains and capillary has not run dry
- May be electrical voltage spike; rerun sample

DNA Interpretation: CE Run Issues

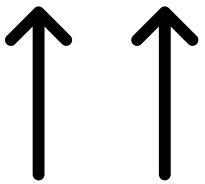
Peak broadening

- Capillary is failing
- Replace capillary

DNA Interpretation: Signal Intensities

Too High

- Reduce DNA quantity inputted in PCR
- Reduce the quantity of DNA inputted for CE from 1 ng
- Reinject from 25 s down to 15 s and 5 s injection time

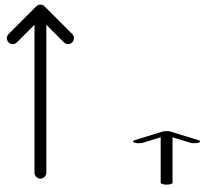


Too Low

- Purify the PCR products (e.g., EZ1 cartridge)
- Concentrate the PCR products (e.g., ethanol precipitation)
- Perform WGA to enrich template prior to PCR or reamplify with a higher input of DNA
- Decrease the quantity of size standard to peak heights of approx. 500 RFU
- Increase the injection time
- Input more ladder if peak is below detection value

Imbalanced

- Incorrect volume of primer or master mix or primer not well vortexed; reamplify



General Equipment Failure

- **Electrical**
  - Wire connections
  - Power source or surge
- **Mechanical**
  - Metal fatigue (e.g., crack)
  - Lubricant starvation (e.g., stuck parts)
- **Optical**
  - Alignment
    - Realign and reduce vibration
  - Chromatic aberration (e.g., light source variations)

SalmanOgli A, Farhadnia F, Piskin E. Separation by nanoparticles plasmonic resonance with low stress in microfluidics channel (analytical and design). *IET Nanobiotechnol.* **2016** Aug;10(4):230-6. doi: 10.1049/iet-nbt.2015.0067.

General Equipment Failure

- Call instrument technical support or repair service unless you are trained to service the instruments
  - Consult your technical leader or lab director for guidance
  - Your lab may have service plans that cover instrument repair and maintenance
  - In cases of instrument failure, laboratories may choose to replace rather than repair

Study Questions

- Why is it important to use controls and standards in DNA typing?
- List some issues that may lead to little to no amplification.
- What could happen if the stated ramp rate is not used?
- What are some symptoms of spectral failure?
- How can you overcome resolution failure?
- What can you do if the peaks are below the analytical threshold on an electropherogram?

Suggested Readings

- ANSI/ASB Standard 115, Standard for Training in Forensic Short Tandem Repeat Typing Methods using Amplification, DNA Separation, and Allele Detection. 2020. 1st Ed. https://www.aafs.org/sites/default/files/media/documents/115_Std_e1.pdf
- Butler, J.M. Ch 11: Low-Level DNA Testing: Issues, Concerns, and Solutions, Advanced Topics in Forensic DNA Typing: Methodology, Elsevier, 2011.
- FBI, Quality Assurance Standards for DNA Databasing Laboratories, effective July 1, 2020.
- FBI, Quality Assurance Standards for Forensic DNA Testing Laboratories, effective July 1, 2020.